## DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9-8-10 has been entered.

Applicant's amendment filed 7-9-10 has been entered. Claims 8 and 17 have been amended. Claim 19 has been added. Applicant's amendment filed 9-8-10 has been entered. Claims 8, 18 and 19 have been amended. Claims 1-7 have been canceled. Claims 20-28 have been added. Claims 8, 9, 11, 13-15 and 17-28 are pending and under consideration.

### *Double Patenting*

2. Applicant is advised that should claim 14 be found allowable, claim 20 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

### *Claim Rejections - 35 USC § 112*

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 22-28 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The phrase "in which the growth factor is PDGF-BB" in claims 22-28 is vague and renders the claims indefinite. Claims 22-28 depend from claims 8, 19 or 20 where growth factor is not the only migration-enhancing factor to be selected from. Alpha-thrombin and hyaluronic acid are not growth factors. It is unclear whether alpha-thrombin and hyaluronic acid are also intended in claims 22-28.

### *Claim Rejections - 35 USC § 112*

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 8, 9, 11, 13-15 and 17-28 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for localizing mesenchymal stem cells to an injury site in a patient by administering both mesenchymal stem cells and the recited migration-enhancing factor directly to the injury site, does not reasonably provide enablement for localizing mesenchymal stem cells to an injury site in a patient by administering both mesenchymal stem cells and the recited migration-enhancing factor to the patient via various administration routes. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

While determining whether a specification is enabling, one considered whether the claimed invention provides sufficient guidance to make and use the claimed invention, if not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirement, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (In re Wands, 858 F.2d at 737, 8 USPQ2d 1400, 1404 (Fed. Cir.1988)).

Furthermore, the USPTO does not have laboratory facilities to test if an invention with function as claimed when working examples are not disclosed in the specification, therefore, enablement issues are raises and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The claims are directed to a method of localizing mesenchymal stem cells to an injury site comprising administering to a patient mesenchymal stem cells and a mesenchymal stem cell migration-enhancing factor, thereby enhancing the migration and accumulation or suppressing the diffusion of the administered mesenchymal stem cells in the injured tissue, wherein the migration-enhancing factor is EGF, PDGF-BB, HB-EGF, hyaluronic acid or bFGF. Claim 9 specifies the factor is administered simultaneously with, or continuously to, or separately from mesenchymal stem cells. Claim 11 specifies the injured tissue results from osteoarthritis, bone

fracture etc. Claims 13-15 specify the factor is administered to the injury site or the periphery thereof, by injection and applied over the injured tissue, respectively. Claim 19 specifies the mesenchymal stem cell migration-enhancing factor is administered as a complex with atelocollagen by injection into the injured tissue. Claim 21 specifies the factor is administered by injection of a transplant comprising said factor. Claims 22-28 specify the growth factor is PDGF-BB.

The specification discloses that PDGF-BB, bFGF, HB-EGF, TGF-alpha, PDGF-AB, IGF-I, EGF, alpha-thrombin and HGF can enhance the migration and proliferation of the rabbit-derived mesenchymal stem cells in vitro (e.g. Examples 2-3). GFP-MSCs injected through the tail vein of rats can migrate to the calves or migrate and accumulate in greater amounts at the site where PDGF-BB was localized (Example 4). The specification fails to provide adequate guidance and evidence for how to localize mesenchymal stem cells to an injury site in a patient by administering both mesenchymal stem cells and migration enhancing factor to the patient via various administration routes.

The claims read on administering mesenchymal stem cells and the recited migration-enhancing factors (proteins or peptides) to a patient via various administration routes such that the mesenchymal stem cells are localized to an injury site in said patient. The art of delivering a protein complex to various target sites in vivo was unpredictable at the time of the invention. The administration route includes direct injection or application, subcutaneous, intravenous, intramuscular, intraperitoneal, oral, topical, dermal, transdermal, and intranasal administration etc. There are various barriers before a protein can reach its target cells, for example, layers of dermal cells, blood vessel wall cell membranes, proteases and lysosomal degradation within

cells, extracellular matrix between cells, gastrointestinal digestive acids, and blood-brain barrier for reaching cells in the brain. Whether the protein can reach target cells in vivo or not depends on the administration route of said protein. Hamman et al., 2005 (Biodrugs, Vol. 19, No. 3, p. 165-177) points out problems with oral administration of peptide or protein drugs. "The main reasons for the low oral bioavailability of peptide drugs are pre-systemic enzymatic degradation and poor penetration of the intestinal mucosa" (e.g. abstract). Barriers limiting the oral bioavailability of peptide drugs include physical barrier, such as cell membranes and tight junctions between adjacent epithelial cells, mucus layer and efflux system, enzymatic barrier, fast elimination from the systemic circulation, the potential to develop an immune response, uptake by non-target tissues, and inefficient target cell entry (e.g. p. 166, right column). The peptide drugs administered via administration routes other than oral administration also would encounter the physical barriers as discussed and above, the enzymatic barrier, potential to develop immune response, and uptake by non-target tissues. Torchilin et al., 2003 (DDT, Vol. 8, No. 6, p. 259-266) discusses the problems of protein delivery in vivo. "The use of protein and peptide as therapeutic agents is hampered by their rapid elimination from the circulation through renal filtration, enzymatic degradation, uptake by the reticuloendothelial system (RES) and accumulation in non-targeted organs and tissues" (e.g. p. 259, right column, last paragraph). Similarly, delivery of mesenchymal stem cells to an injury site via various administration routes also would encounter various barriers in vivo, such as cell membranes and tight junctions between adjacent epithelial cells, mucus layer and efflux system, enzymatic barrier, fast elimination from the systemic circulation, the potential to develop an immune response, uptake by non-target tissues, and inefficient target cell entry. There is no evidence of record that

demonstrates administration of the mesenchymal stem cells and the claimed factors via various administration routes would be able to localize the mesenchymal stem cells to a target injury site.

The specification discloses administration of GFP-MSCs via tail vein can localize the GFP-MSCs to where PDGF-BB is injected in rats (e.g. p. 27). It appears that Example 4 of the specification shows the accumulation of injected GFP-MSCs to the injected site of PDGF-BB. However, the specification fails to provide evidence for whether the PDGF-BB can be accumulated to various injury sites in a patient via various administration routes, including intravenous, subcutaneous, intramuscular, oral, and intraperitoneal administration etc. As discussed above, there are physical barriers, including layers of dermal cells, blood vessel wall cell membranes, proteases and lysosomal degradation within cells, extracellular matrix between cells, gastrointestinal digestive acids, and blood-brain barrier for reaching cells in the brain etc, and the enzymatic barrier, potential to develop immune response, and uptake by non-target tissues. There is no evidence of record that shows the PDGF-BB can reach various injury sites in a patient via various administration routes. Absent specific guidance, one skilled in the art at the time of the invention would not know how to localize mesenchymal stem cells to an injury site in a patient by administering both mesenchymal stem cells and migration-enhancing factor via various administration routes.

For the reasons discussed above, it would have required undue experimentation for one skilled in the art at the time of the invention to practice over the full scope of the invention claimed. This is particularly true given the nature of the invention, the state of the prior art, the breadth of the claims, the amount of experimentation necessary, the level of skill which is high,

the working examples provided and scarcity of guidance in the specification, and the unpredictable nature of the art.

***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 8, 9, 11, 13-15, 20-25, 27 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over each of Fiedler et al., 2002 (Journal of Cellular Biochemistry, Vol. 87, p. 305-312), Gerber et al., 2002 (US 20020132978 A1), Badylak et al., 2002 (US Patent No. 6,375,989 B1), Desnoyers et al., 2008 (US Patent No. 7,456,262 B2), or Dabbagh et al., 1998 (Thrombosis and Haemostasis, Vol. 79, No. 2, pp. 405, Summary only).

The claims are directed to a method of localizing mesenchymal stem cells to an injury site comprising administering to a patient mesenchymal stem cells and a mesenchymal stem cell

migration-enhancing factor, thereby enhancing the migration and accumulation or suppressing the diffusion of the administered mesenchymal stem cells in the injured tissue, wherein the migration-enhancing factor is EGF, PDGF-BB, HB-EGF, hyaluronic acid or bFGF. Claim 9 specifies the factor is administered simultaneously with, or continuously to, or separately from mesenchymal stem cells. Claim 11 specifies the injured tissue results from osteoarthritis, bone fracture etc. Claims 13-15 specify the factor is administered to the injury site or the periphery thereof, by injection and applied over the injured tissue, respectively. Claim 21 specifies the factor is administered by injection of a transplant comprising said factor. Claims 22-25, 27 and 28 specify the growth factor is PDGF-BB.

Fiedler discloses that human platelet derived growth factor bb (rhPDGF-bb) can stimulate migration of primary human mesenchymal progenitor cells (MPC) in a dose-dependent manner. The effect of rhPDGF-bb as chemoattractive proteins for primary human MPC suggests a functional role for recruitment of MPCs during bone development and remodeling, as well as fracture healing (e.g. abstract). The mesenchymal progenitor cell is a type of mesenchyma stem cell.

Gerber teaches that the growth factor HB-EGF stimulates mesenchymal cell proliferation and migration and promotes renal epithelial cell survival (e.g. [0055]). The mesenchymal cell is "an undifferentiated cell found in mesenchyme and capable of differentiating into various specialized connective tissues" (Answers.com, mesenchymal cell). A mesenchymal cell is also a mesenchymal stem cell.

Badylak teaches growth factors FGF-2 and TGF-beta have been identified as particularly important to wound healing and tissue remodeling. FGF-2 promotes mesenchymal cell

migration and proliferation to accelerate healing of gastric mucosa and calvarian bone (e.g. bridging columns 15 and 16). The mesenchymal cell is "an undifferentiated cell found in mesenchyme and capable of differentiating into various specialized connective tissues" (Answers.com, mesenchymal cell). A mesenchymal cell is also a mesenchymal stem cell.

Desnoyers teaches that hyaluronic acid (HA) is a component of skin and mesenchymal tissue where it facilitates cell migration during wound healing (e.g. bridging columns 3 and 4). The mesenchymal cell is "an undifferentiated cell found in mesenchyme and capable of differentiating into various specialized connective tissues" (Answers.com, mesenchymal cell). A mesenchymal cell is also a mesenchymal stem cell.

Dabbagh teaches that thrombin can stimulate mesenchymal cell migration, proliferation and has been implicated both in normal wound healing and pathological conditions associated with hyperproliferation of smooth muscle cells. The mesenchymal cell is "an undifferentiated cell found in mesenchyme and capable of differentiating into various specialized connective tissues" (Answers.com, mesenchymal cell). A mesenchymal cell is also a mesenchymal stem cell.

It is noted that administration to the injury site or the periphery thereof, by injection and applied over the injured tissue encompass administration directly into the injured site or tissue.

Fiedler, Gerber, Badylak, Desnoyers and Debbagh do not specifically teach administration of rhPDGF-bb, HB-EGF, FGF-2 or HA to injured tissue for localizing mesenchymal stem cells to an injury site, or the mesenchymal stem cells and the factors are administered simultaneously, continuously or separately.

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to use the rhPDGF-bb, HB-EGF, FGF-2 or HA for localizing mesenchymal stem cells to the injured tissue because Fiedler teaches that rhPDGF-bb can stimulate migration of primary human mesenchymal progenitor cells (MPC) in a dose-dependent manner, Gerber teaches HB-EGF stimulates mesenchymal cell proliferation and migration and promotes renal epithelial cell survival, Badylak teaches FGF-2 promotes mesenchymal cell migration and proliferation to accelerate healing of gastric mucosa and calvarian bone, Desnoyers teaches that hyaluronic acid (HA) facilitates cell migration during wound healing, and Dabbagh teaches that thrombin can stimulate mesenchymal cell migration and proliferation, and it would be obvious for one of ordinary skill in the art to administer both mesenchymal stem cells and the migration-enhancing factors to the injury site or surrounding area to localize the mesenchymal stem cells to the injury site. It also would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to administer the claimed factor at different time schedule because determining effective administration schedule of the factor is routine optimization of a result-effective variable and is obvious to a person of ordinary skill.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to use the factor for recruitment of MPCs during bone development and remodeling, as well as fracture healing as taught by Fiedler or to use the factor for wound healing and tissue remodeling as taught by Badylak with reasonable expectation of success.

10. Claims 19 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over each of Fiedler et al., 2002 (Journal of Cellular Biochemistry, Vol. 87, p. 305-312), Gerber et al., 2002

(US 20020132978 A1), Badylak et al., 2002 (US Patent No. 6,375,989 B1), Desnoyers et al., 2008 (US Patent No. 7,456,262 B2), or Dabbagh et al., 1998 (Thrombosis and Haemostasis, Vol. 79, No. 2, pp. 405, Summary only) as applied to claims 8, 9, 11, 13-15, 20-25, 27 and 28 above, and further in view of Sano et al., 2003 (Advanced Drug Delivery Reviews, Vol. 55, p. 1651-1677).

The claims are directed to a method of localizing mesenchymal stem cells to an injury site comprising administering to a patient mesenchymal stem cells and a mesenchymal stem cell migration-enhancing factor, thereby enhancing the migration and accumulation or suppressing the diffusion of the administered mesenchymal stem cells in the injured tissue, wherein the migration-enhancing factor is EGF, PDGF-BB, HB-EGF, hyaluronic acid or bFGF, wherein the migration-enhancing factor is administered as a complex with atelocollagen by injection into the injured tissue. Claim 26 specifies the growth factor is PDGF-BB.

The teachings of Fiedler, Gerber, Badylak, Desnoyers and Debbagh are as discussed above. Fiedler, Gerber, Badylak, Desnoyers and Debbagh do not specifically teach the mesenchymal stem cell migration-enhancing factor is complexed with atelocollagen for injection into the injured tissue.

Sano teaches using atelocollagen as a protein carrier for sustained release of protein in vivo. Atelocollagen administered into the living body is not dissolved immediately but exists for a long time (e.g. p. 1654, left column).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to complex the mmigration-enhancing factor with atelocollagen for delivery to a

subject because Sano teaches using atelocollagen as a protein carrier for sustained release of protein in vivo.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to provide a sustained release of the protein in vivo as taught by Sano with reasonable expectation of success.

Applicant argues that the claims relate to homing of mesenchymal stem cells and the specification provides in vitro data showing the recited growth factors have chemoattractant activity for mesenchymal stem cells. The in vitro data of Examples 2 and 3 are predictive of the results in vivo and Example 4 demonstrates the PDGF-BB is effective in vivo (amendment, p. 5-6). This is not found persuasive because of the reasons set forth above under 35 U.S.C., first paragraph. It appears that Example 4 of the specification shows the accumulation of injected GFP-MSCs to the injected site of PDGF-BB. However, the specification fails to provide evidence for whether the PDGF-BB can be accumulated to various injury sites in a patient via various administration routes, including intravenous, subcutaneous, intramuscular, oral, and intraperitoneal administration etc. There are physical barriers for protein delivery in vivo, including layers of dermal cells, blood vessel wall cell membranes, proteases and lysosomal degradation within cells, extracellular matrix between cells, gastrointestinal digestive acids, and blood-brain barrier for reaching cells in the brain etc, and the enzymatic barrier, potential to develop immune response, and uptake by non-target tissues. There is no evidence of record that shows the PDGF-BB can reach various injury sites in a patient via various administration routes. Absent specific guidance, one skilled in the art at the time of the invention would not know how

to localize mesenchymal stem cells to an injury site in a patient by administering both mesenchymal stem cells and migration-enhancing factor via various administration routes.

***Conclusion***

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shin-Lin Chen whose telephone number is (571) 272-0726. The examiner can normally be reached on Monday to Friday from 9:30 am to 6 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for this group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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